METHODS AND COMPOSITIONS FOR DIAGNOSIS AND TREATMENT OF B CELL CHRONIC LYMPHOCYTIC LEUKEMIA

Cross-Reference to Related Application

This application claims the benefit of U.S. Provisional Application No. 60/509,473, filed Oct. 8, 2003.

Statement Regarding Federally Funded Research or Development

The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided by the terms of Grants No. CA 81554 and CA 87956 awarded by the National Institutes of Health.

Background

The present invention generally relates to methods of diagnosis and treatment of B cell chronic lymphocytic leukemia (B-CLL). More particularly, the invention relates to methods of B-CLL diagnosis and treatment based on the presence of sets of B-CLL patients that have B cell receptor genes in common.

(2) Description of the Related Art

References cited

Bendelac, A., M. Bonneville, and J.F. Kearney. Autoreactivity by design: innate B and T lymphocytes. Nat Rev Immunol. 2001:1:177-186.

Borche L et al. Blood 1990; 76:562-569.

Brezinschek HP, Foster SJ, Brezinschek RI, Dorner T, Domiati-Saad R, Lipsky PE. Analysis of the human VH gene repertoire. Differential effects of selection and somatic hypermutation on human peripheral CD5(+)/IgM+ and CD5(-)/IgM+ B cells. J Clin Invest. 1997;99:2488-2501.

Broker BM et al. J Autoimmun 1988; 1:469-481.

Casali P, Schettino EW. Structure and function of natural antibodies. Current Topics in Microbiology & Immunology. 1996;210:167-179.

Chapman CJ, Spellerberg MB, Smith GA, Carter SJ, Hamblin TJ, Stevenson FK. Autoanti-red cell antibodies synthesized by patients with infectious mononucleosis utilize the VH4-21 gene segment. Journal of Immunology 1993;151:1051-1061.

Chiorazzi, N., and M. Ferrarini. Immunoglobulin Variable Region Gene Characteristics and Surface Membrane Phenotype Define B-CLL Subgroups with Distinct Clinical Courses. *In* Chronic Lymphoid Leukemias. B.D. Cheson, editor. Marcel Dekker, New York. 2001;81-109.

Chiorazzi, N., and M. Ferrarini. B Cell Chronic Lymphocytic Leukemia:Lessons Learned from Studies of the B Cell Antigen Receptor. *In* Annual Review of Immunology. W.E. Paul, editor. 2003; 21:841-894.

Damle, R.N., T. Wasil, F. Fais, F. Ghiotto, A. Valetto, S.L. Allen, A. Buchbinder, D. Budman, K. Dittmar, J. Kolitz, S.M. Lichtman, P. Schulman, V.P. Vinciguerra, K.R. Rai, M. Ferrarini, and N. Chiorazzi. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood. 1999;94:1840-1847.

Fais F, Ghiotto F, Hashimoto S, Sellars B, Valetto A, Allen SL, Schulman P, Vinciguerra VP, Rai K, Rassenti LZ, Kipps TJ, Dighiero G, Schroeder HW, Jr., Ferrarini M, Chiorazzi N. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. J Clin Invest. 1998;102:1515-1525.

Fais, F., G. Gaidano, D. Capello, A. Gloghini, F. Ghiotto, S. Roncella, A. Carbone, N. Chiorazzi, and M. Ferrarini. Immunoglobulin V region gene use and structure suggest antigen selection in AIDS-related primary effusion lymphomas. Leukemia 1999;13:1093-1099.

Geiger, K.D., U. Klein, A. Brauninger, S. Berger, K. Leder, K. Rajewsky, M.L. Hansmann, and R. Kuppers. CD5-positive B cells in healthy elderly humans are a polyclonal B cell population. Eur J Immunol. 2000;30:2918-2923.

Ghia, P., G. Prato, C. Scielzo, S. Stella, M. Geuna, G. Guida, and F. Caligaris-Cappio. Monoclonal CD5+ and CD5- B-lymphocyte expansions are frequent in the peripheral blood of the elderly. Blood. 2004;103:2337-2342.

Ghiotto, F., F. Fais, A. Valetto, E. Albesiano, S. Hashimoto, M. Dono, H. Ikematsu, S.L. Allen, K.R. Rai, M. Nardini, A. Tramontano, M. Ferrarini, and N. Chiorazzi. Remarkable similar antigen receptors among a subset of patients with chronic lymphocytic leukemia. J Clin Invest. 2004; 113:1008-1016.

Hamblin, T.J., Z. Davis, A. Gardiner, D.G. Oscier, and F.K. Stevenson. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999;94:1848-1854.

Hashimoto, S., M. Wakai, J. Silver, and N. Chiorazzi. Ann. NY. Acad. Sci. 1992; 651:477-479.

He X, Goronzy JJ, Zhong W, Xie C, Weyand CM. VH3-21 B cells escape from a state of tolerance in rheumatoid arthritis and secrete rheumatoid factor. Mol Med. 1995;1:768-780.

Isaacson, P.G. Gastric MALT lymphoma: from concept to cure. Ann Oncol. 1999;10:637-645.

Jahn S, Schwab J, Hansen A, Heider H, Schroeder C, Lukowsky A, Achtman M, Matthes H, Kiessig ST, Volk HD. Human hybridomas derived from CD5+ B lymphocytes of patients with chronic lymphocytic leukemia (B-CLL) produce multi-specific natural IgM (kappa) antibodies. Clin Exp Immunol. 1991;83:413-417.

Johnson TA, Rassenti LZ, Kipps TJ. Ig VH1 genes expressed in B cell chronic lymphocytic leukemia exhibit distinctive molecular features. J Immunol. 1997;158:235-246.

Kirkham, P.M., F. Mortari, J.A. Newton, and H.W. Schroeder, Jr. EMBO. J. 1997;11:603-609.

Kipps TJ, Tomhave E, Pratt LF, Duffy S, Chen PP, Carson DA. Developmentally restricted immunoglobulin heavy chain variable region gene expressed at high frequency in chronic lymphocytic leukemia. Proc Natl Acad Sci USA. 1989;86:5913-5917.

Kumar, S., S. Nagl, J.K. Kalsi, C.T. Ravirajan, D. Athwal, D.S. Latchman, L.H. Pearl, and D.A. Isenberg. Anti-cardiolipin/beta-2 glycoprotein activities co-exist on human anti-DNA antibody light chains. Mol Immunol. 2003;40:517-530.

Mann, D.L., P. DeSantis, G. Mark, A. Pfeifer, M. Newman, N. Gibbs, M. Popovic, M.G. Sarngadharan, R.C. Gallo, J. Clark, and et al. HTLV-I--associated B-cell CLL: indirect role for retrovirus in leukemogenesis. Science. 1987;236:1103-1106.

Martin, F., and J.F. Kearney. B-cell subsets and the mature preimmune repertoire. Marginal zone and B1 B cells as part of a "natural immune memory". Immunol Rev. 2000;175:70-79.

Pascual, V., K. Victor, M. Spellerberg, T.J. Hamblin, F.K. Stevenson, and J.D. Capra. VH restriction among human cold agglutinins. The VH4-21 gene segment is required to encode anti-I and anti-i specificities. J Immunol. 1992;149:2337-2344.

Potter, M. Antigen-binding myeloma proteins of mice. Adv Immunol. 1977;25:141-211.

Pugh-Bernard AE, Silverman GJ, Cappione AJ, Villano ME, Ryan DH, Insel RA, Sanz I. Regulation of inherently autoreactive VH4-34 B cells in the maintenance of human B cell tolerance. The Journal of Clinical Investigation. 2001;108:1061-1070.

Radic, M.Z., and M. Weigert. Genetic and structural evidence for antigen selection of anti-DNA antibodies. Annu Rev Immunol. 1994;12:487-520.

Rawstron, A.C., M.J. Green, A. Kuzmicki, B. Kennedy, J.A. Fenton, P.A. Evans, S.J. O'Connor, S.J. Richards, G.J. Morgan, A.S. Jack, and P. Hillmen. Monoclonal B lymphocytes with the characteristics of "indolent" chronic lymphocytic leukemia are present in 3.5% of adults with normal blood counts. Blood. 2002;100:635-639.

Schroeder HWJ, Dighiero G. The pathogenesis of chronic lymphocytic leukemia: analysis of the antibody repertoire [see comments]. Immunol Today. 1994;15:288-294.

Scott, M.G., D.L. Crimmins, D.W. McCourt, I. Zocher, R. Thiebe, H.G. Zachau, and M.H. Nahm. Clonal characterization of the human IgG antibody repertoire to Haemophilus influenzae type b polysaccharide. III. A single VKII gene and one of several JK genes are joined by an invariant arginine to form the most common L chain V region. J Immunol. 1989;143:4110-4116.

Seidl, K.J., J.D. MacKenzie, D. Wang, A.B. Kantor, E.A. Kabat, and L.A. Herzenberg. Frequent occurrence of identical heavy and light chain Ig rearrangements. Int Immunol. 1997;9:689-702.

Silverman, G.J., R.D. Goldfien, P. Chen, R.A. Mageed, R. Jefferis, F. Goni, B. Frangione, S. Fong, and D.A. Carson. Idiotypic and subgroup analysis of human monoclonal rheumatoid factors. Implications for structural and genetic basis of autoantibodies in humans. J Clin Invest. 1988;82:469-475.

Smith G, Spellerberg M, Boulton F, Roelcke D, Stevenson F. The immunoglobulin VH gene, VH4-21, specifically encodes autoanti-red cell antibodies against the I or i antigens. Vox Sang. 1995;68:231-235.

Stevenson FK, Spellerberg MB, Chapman CJ, Hamblin TJ. Differential usage of an autoantibody-associated VH gene, VH4-21, by human B-cell tumors. Leukemia & Lymphoma. 1995;16:379-384.

Sthoeger, Z.M., M. Wakai, D.B. Tse, V.P. Vinciguerra, S.L. Allen, D.R. Budman, S.M. Lichtman, P. Schulman, L.R. Weiselberg, and N. Chiorazzi. Production of autoantibodies by CD5-expressing B lymphocytes from patients with chronic lymphocytic leukemia. J Exp Med. 1989;169:255-268.

Sthoeger ZM et al. Am J Hematol 1993; 43:259-264.

Tobin, G., U. Thunberg, A. Johnson, I. Thorn, O. Soderberg, M. Hultdin, J. Botling, G. Enblad, J. Sallstrom, C. Sundstrom, G. Roos, and R. Rosenquist. Somatically mutated Ig V(H)3-21 genes characterize a new subset of chronic lymphocytic leukemia. Blood. 2002;99:2262-2264.

Tobin G. et al. Blood 2003; 101:4952-4957.

Wakai M, Hashimoto S, Omata M, Sthoeger ZM, Allen SL, Lichtman SM, Schulman P, Vinciguerra VP, Diamond B, Dono M, et al. IgG+, CD5+ human chronic lymphocytic leukemia B cells. Production of IgG antibodies that exhibit diminished autoreactivity and IgG subclass skewing. Autoimmunity. 1994;19:39-48.

Zemlin, M., M. Klinger, J. Link, C. Zemlin, K. Bauer, J.A. Engler, H.W. Schroeder, Jr., and P.M. Kirkham. Expressed murine and human CDR-H3 intervals of equal length exhibit

distinct repertoires that differ in their amino acid composition and predicted range of structures. J Mol Biol. 2003;334:733-749.

B cell chronic lymphocytic leukemia (B-CLL) is an accumulative disease of slowly proliferating $CD5^+$ B lymphocytes that develops in the aging population. Whereas some patients with B-CLL have an indolent course and die after many years from unrelated causes, others progress very rapidly and succumb within a few years from this currently incurable leukemia. Over the past decade, studies of the structure and function of the B cell antigen receptor (BCR) used by these leukemic cells have helped redefine the nature of this disease.

$CD5^+$ B lymphocytes in B-CLL patients express low levels of surface membrane Ig that serves as their receptor for antigen (BCR). The genetics of this Ig have clinical relevance, as patients with an Ig that is unmutated in the variable (V) regions have a significantly worse outcome than those with significant numbers of mutations in the Ig V region. The biological basis by which the Ig molecule/BCR associates with these distinct outcomes is unclear.

There are several lines of evidence supporting a role for the Ig molecule in the evolution of B-CLL. Analysis of V region gene cassette usage has provided inferential evidence that the Ig molecules on B-CLL cells are not the product of random chance. The distribution of variable region gene cassettes used by B-CLL clones (Schroeder and Dighiero, 1994) differs from that found in normal cells (Brezinschek et al., 1997) with an increased frequency of V_H 3-07, V_H 4-34, and V_H 1-69 genes (Fais et al., 1998). Furthermore, the distribution of mutations among B-CLL cases using these specific V_H genes is selectively and strikingly biased. For instance, the V_H genes of ~40% of B-CLL cases contain <2% differences from the most similar germline gene and ~25% are identical to a germline V_H counterpart. However, 80% of the cases that use a V_H 1-69 are germline and ~90% of these have less than 2% mutation. Conversely, in 93% of cases the V_H 3-07 gene exhibits significant numbers of mutations (≥2% difference from the germline gene). These deviations from randomness in gene use and acquisition of somatic mutations imply that the structure of the antibody molecule, and possibly its antigen specificity thus manifest, played a role in the leukemic transformation of particular B cells.

More recently, sets of B-CLL cases with highly similar Ig molecules have been identified. Our laboratory identified five unmutated IgG-expressing B-CLL cases in which the BCR was remarkably similar in structure (Ghiotto et al. 2003). These Ig molecules used the same V_H, D, J_H, and in all but one instance the same V_K-J_K. Furthermore, the HCDR3s were highly similar in sequence and the LCDR3s were virtually identical with a V_K-J_K junction contained an invariant, non-templated arginine codon. A larger set of patients expressing a V_H3-21/J_H3 H chain and a $V\lambda$-3h/$J\lambda$3 L chain have been described by Tobin et al. (2003). These cases also have a HCDR3 that is small and of very similar sequence. The VH3-21 gene is not found at high frequency

outside of northern Europe, suggesting an environmental or genetic influence. The patients from both of these groups have a poor clinical course that does not necessarily relate to their VH mutation status.

Functional studies have shown that patients with unmutated Ig V regions can transduce signals through the B cell receptor (BCR), while the mutated BCR cannot. This finding could have major significance since it provides a means by which antigen binding to the BCR might affect the biology of the leukemic cells in vivo. This is especially relevant since many B-CLL cases synthesize autoreactive Ig/BCR molecules (Broker et al., 1988; Borche et al., 1990; Sthoeger et al., 1993) and/or use VH genes that are often found in autoantibodies (Fais et al., 1998). This is consistent with the derivation of the leukemic cells from $CD5^+$ B-cells that in normal individuals are considered the primary source of natural antibodies (Casali and Schettino, 1996).

Despite recent identification of several biomarkers associated with outcome in B-CLL, there is a need for additional prognostic indicators for this disease. Also, there is a long-standing need for therapeutic targets and new therapeutic modalities in B-CLL, for which there is no generally accepted and specific curative regimen. The present invention addresses these needs.

Summary of the Invention

Accordingly, the inventors have discovered that the B-CLL cells of a significant proportion of B-CLL patients with an aggressive form of the disease share the same classes of V_H, D, J_H, V_L, and J_L antibody genes as other B-CLL patients, forming "sets" of B-CLL patients with highly homologous B cell receptors. This discovery makes practical various therapeutic and diagnostic methods.

Thus, in some embodiments, the invention is directed to isolated and purified preparations of a combination of a light chain antibody gene and a heavy chain antibody gene. In these preparations, the family members of the light chain antibody gene and the heavy chain antibody gene are selected from the group consisting of V_H4-39/D6-13/J_H5/$V_L\kappa$O12/2/$J_L\kappa$1/κ2 (Set I), V_H4-34/D5-5/J_H6/$V_L\kappa$A17/$J_L\kappa$1/κ2 (Set II), V_H3-21/J_H6/$V_L\lambda$3h/$J_L\lambda$3 (Set III), V_H1-69/D3-16/J_H3/$V_L\kappa$A27/$J_L\kappa$1/κ4 (Set IV), V_H1-69/D3-10/J_H6/$V_L\lambda$1c/$J_L\lambda$1 (Set V), V_H1-02/D6-19/J_H4/$V_L\kappa$O12/2/$J_L\kappa$1/κ2 (Set VIa), V_H1-03/D6-19/J_H4/$V_L\kappa$O12/2/$J_L\kappa$1/κ2 (Set VIb), V_H1-18/D6-19/J_H4/$V_L\kappa$O12/2/$J_L\kappa$1 (Set VIc), V_H1-46/D6-19/J_H4 (Set VId), V_H5-51/D6-19/J_H4/$V_L\kappa$O12/2/$J_L\kappa$2 (Set VIe), V_H1-69/D3-3/J_H4/$V_L\kappa$A19/$J_L\kappa$4 (Set VII), and V_H1-69/D2-2/J_H6/$V_L\kappa$L6/2/$J_L\kappa$3 (Set VIII).

The invention is also directed to cells in culture comprising at least one vector comprising antibody genes from Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII.

In other embodiments, the invention is directed to isolated and purified antibodies encoded by antibody genes from Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII.

In further embodiments, the invention is directed to anti-idiotype antibodies that bind to the antigen-binding region of an antibody encoded by antibody genes from Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII.

The invention is additionally directed to hybridomas expressing any of the above-described antibodies.

In related embodiments, the invention is directed to bispecific antibodies comprising the binding site of the above-described anti-idiotype antibodies and a binding site that binds to another B-cell antigen.

The present invention is additionally directed to peptide antigens that bind to the antigen-binding region of an antibody encoded by antibody genes of Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII.

In further embodiments, the invention is directed to aptamers that bind to the antigen-binding region of an antibody encoded by antibody genes of of Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII.

The present invention is also directed to multimeric molecules comprising at least a first and a second binding site. In these embodiments, the first binding site binds to the antigen-binding region of an antibody encoded by antibody genes of Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII, and the second binding site binds to either (a) the antigen-binding region of an antibody encoded by antibody genes of Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII or (b) a B-cell antigen.

The invention is additionally directed to isolated and purified preparations of a combination of a light chain antibody gene and a heavy chain antibody gene. In these embodiments, the gene family members of the light chain antibody gene and the heavy chain antibody gene are present in B cells of two or more patients, and the antibody chains of the B cells also share the same isotype, JH, D and JL regions, and the B cells are lymphoproliferative in the patient, or the patient has an autoimmune disease involving the B cells.

In other embodiments, the invention is directed to methods of determining whether a patient with B cell chronic lymphocytic leukemia (B-CLL) has a form of B-CLL that is

susceptible to treatment directed to eliminating idiotype-specific B cell receptor-bearing B-CLL cells. The method comprises determining whether the B cell receptors on the patient's B-CLL cells have an idiotype encoded by antibody genes from Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII.

In related embodiments, the present invention is directed to methods of following the progression of treatment of B-CLL in the patient identified by the above-described method as having a form of B-CLL susceptible to treatment directed to eliminating idiotype-specific B cell receptor-bearing B-CLL cells. The methods comprise determining whether the B cell receptors on the B-CLL cells have an idiotype encoded by antibody genes from Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII.

In further embodiments, the invention is directed to methods of treating a patient having B-CLL, where the B-CLL is caused by B cells comprising antibody genes from Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId or Set VIe, Set VII, or Set VIII. The methods comprise administering to the patient any of the anti-idiotype antibodies, peptide antigens, or aptamers described above, or mixtures thereof.

In additional embodiments, the invention is directed to methods of identifying a B-CLL set. The methods comprise identifying the VH, D, JH, VL, and JL antibody gene families present on B-CLL cells, where the same antibody gene families are all present in more than one B-CLL patient.

Brief Description of the Drawings

FIG. 1 provides VH, D and JH regions of antibody genes from B-CLL cells of Sets I-VIe.

FIG. 2 shows amino acid alignments of the H chain V regions of all sequences in Sets II, IV, V, VIa-e, and VIII. A period indicates homology with the germline gene. Amino acids in gray are chemically similar to the germline-encoded residues. Underlined positions are known sites of allelic polymorphism. The consensus sequence for the set is shown at the bottom of each alignment.

FIG. 3 shows amino acid alignments of the L chain variable regions of all sequences in Sets II, IV, V, VI, and VIII. See FIG. 2 description above.

FIG. 4 shows amino acid and nucleotide sequences of the CDR3 and its junctions of set IV. The H chain sequences are shown at left, and the L chain sequences are shown at right. The most similar germline genes are shown at top. Dots indicate homology with the germline sequence. Dashes indicate no sequence at that position. The numbering at bottom is for convenience of reference and is arbitrary. Sequences from the public databases have their GenBank accession number in parenthesis below the case ID. Distinctive junctional residues

exist, including a pair of G codons at the VH-D junction and an N codon at the D-JH junction. The creation of the G codon at the VH-D junction required trimming of the 3' adenosine nucleotide at the end of *IgVH*, along with N addition. Also, limited trimming at the 5' end of the *D* segment eliminated the first of the pair of Y codons in all cases. In two instances, D replaced Y and in two other cases N does the same; both of these are charged residues that fit at the negative end of the Kyte-Doolittle scale. The Y codon at the 3' end of the *D* gene was also eliminated in all sequences of this set. Collectively, these conserved junctional adjustments suggest strong selection for HCDR3 structure. Three rearranged L chain sequences were available for this set and both contained the *VκA27* gene associated with *Jκ1, Jκ4,* or *Jκ5*.

FIG. 5 shows amino acid and nucleotide sequences of the CDR3 and its junctions of Set VIII. The VH-D junctions are dominated by non-templated Gs. The D-JH junction exhibits evidence of trimming and fill-in, with an alteration to M where the final *D* encoded residue would be found. This is not a known site of polymorphism, although that explanation cannot be excluded. Only one L chain sequence was available for this set (GO13), and this consisted of the *VκL6* and *Jκ3* genes. There was significant overlap between the germline segments at the VL-JL junction.

FIG. 6 shows amino acid and nucleotide sequences of the CDR3 and its junctions of Set V. In these sequences, the 5' end of the germline *D* gene overlaps the 3' end of the germline *IgVH* segment to form the VH-D junction. The presence of several nucleotides that do not match either germline sequence in the overlap region suggests that trimming and addition occurred, resulting in a preferred insertion of a residue with a small (A, S, and V) or no (G) side chain. The amino acids at the D-JH junction are not well conserved. However, the consistent relative positioning of the *VH, D*, and *JH* segments is intriguing because the region of overlap between the *VH* and *D* does not contain significant homology as might be predicted for preferential recombination. This suggests selection for HCDR3 configuration and D-encoded residues rather than specific junctional residues. Two rearranged L chain sequences were available from this set (RF22 and GN12) and both were comprised of *V*λ1.16 (*1c*) and *Jλ1* segments. The level of mutation of both the H and L chains in the members of sets IV, V, and VIII was always <2%, which is consistent with published reports of the frequent lack or scarcity of mutations in the *VH1-69* in B-CLL (Kipps et al., 1989; Schroeder et al., 1994; Fais et al., 1998).

FIG. 7 shows amino acid and nucleotide sequences of the CDR3 and its junctions of Set II. The H chain junctions of the sequences in this set of five cases are quite constrained. The position of the *D* (*D5-5*) relative to both *VH* (*VH 4-34*) and JH (*JH6*) segments is identical for each member, leading to equal HCDR3 lengths. The VH-D and D-JH junctions both contain evidence of trimming and addition. These processes produced an aromatic residue (W, Y, F) at

the VH-D junction (position 5) followed by a hydrophobic residue (G, P, or A at position 6) and a pair of codons encoding basic residues (K or R) at the D-JH junction (positions 12 and 13). At position 9 in the D segment, four out of the five HCDR3 sequences exhibit a P rather than an A found in the canonical *D5-5* segment deposited in the public databases. Although this is most likely a polymorphism of the *D5-5* segment rather than a common mutation, the last of the five sequences in this set (CLL ID47) also deviates from the canonical *D5-5* sequence at this codon, substituting a D. These highly conserved alterations of the VH-D-JH junctions suggest selection for a very particular HCDR3 structure. The rearranged L chains of this set are also very similar. All three available VLJL sequences use *VκA17* and either *Jκ1* or *Jκ2*. The junctions are highly similar with only a single difference that results from an abbreviated recombination that eliminates the junctional P from CLL240. These cases are of the IgG isotype. Like most IgG^+ B-CLL cases that express a switched isotype (Fais et al., , 1998; Hashimoto et al., 1992; Ghiotto et al., 2004), these cases exceed the 2% difference from germline, albeit slightly, and are thus classified as mutated.

FIG. 8 shows amino acid and nucleotide sequences of the CDR3 and its junctions of set VI. The *VH1- 02* germline sequence is shown. There are no sequence differences between *VH1-02* and *VH1-03*, *1-18*, *1-46*, or *5-51* for the displayed region. The *Jκ1* gene is shown, and homology between CLL011 and CLL-412 and *Jκ2* at positions where the germline sequence of *Jκ2* and *Jκ1* are different is indicated with an asterisk. This set is composed of five subsets, totally 22 patients that share HCDR3 and VLJL characteristics but incorporate different *IgVH* genes (*1-02*, *1-03*, *1-18*, *1-46*, and *5-51*). Each of these genes belongs to the same *VH* clan (Kirkham et al., 1992). The HCDR3 of these subsets all share a precise VHD overlap. Curiously, the *D6-19* segment was used in a nonproductive reading frame. However, this stop codon was in the region of overlap with the terminal *IgVH* sequence and was trimmed, thereby allowing productive rearrangements with the *JH4* segment. The D-JH junctions contain evidence for trimming and addition. The first nongermline templated codon after the *D* segment is enriched in redundant L codons, but the remaining junctional codons are not tightly conserved. All the rearranged L chains available for this set use the *Vκ O12/2* gene with *Jκ* use restricted to *Jκ1* and *Jκ2*. Of these 10 sequences, 9 are essentially identical to that of the germline in the LCDR3 and junctional regions. Thus, this set is unified not only by its common HCDR3 structure and motifs but also by the use of a virtually identical VLJL partner with a very restricted LCDR3 composition.

Detailed Description of the Invention

The present invention is based on the discovery that a significant proportion of B-CLL patients having genetic and protein markers consistent with an aggressive form of the disease or a

manifestly aggressive form of the disease regardless of said markers, have B-CLL cells with B cell receptors encoded by antibody gene family members that other B-CLL patients also have. The inventors have identified at least 10 sets of patients (see Table 1 in the Example), where the patients within each set have the same B-CLL B cell receptor antibody genes. This accounts for approximately 10% of B-CLL patients, and about 20% of those patients that have genetic and protein markers consistent with an aggressive form of the disease. See the Example for details relating to the discovery of these sets.

As is known, aggressive forms of B-CLL are correlated with B cells that have relatively few IgV gene mutations and have intercellular expression of ZAP-70, and cell surface expression of CD38 and CD23. These markers are evaluated at first diagnosis to predict which patients will have an aggressive form of the disease, in order to determine a course of treatment. Because the B-CLL cells from patients belonging to identified "sets" with common B cell receptor genes have low or absent IgV mutations (see Table 1 in Example), it is predicted that patients having B-CLL cells from each of these sets will have an aggressive form of the disease.

The Figures provide relevant sequences of the B cell receptor antibodies and antibody genes of B-CLL cells of several patients in the sets. Notable is the relatively small amount of variation within each set in the number of nucleotides added during the VH-D-JH and VL-JL recombinations.

While two of these sets (Sets I and III) have been previously identified, it was believed that those two sets were anomalous and were not expected to account for more than a small fraction of B-CLL cases. Thus, the discovery, disclosed herein, of multiple other sets that account for a significant proportion of patients with B-CLL, in particular the apparently aggressive form of the disease, makes practical the use of various methods and compositions for diagnosis and treatment of B-CLL, based on the sets identified.

Thus, in some embodiments, the present invention is directed to isolated and purified preparations of a combination of a light chain antibody gene and a heavy chain antibody gene. The family members of the light chain antibody gene and the heavy chain antibody gene of these preparations make up any one of the following sets: VH4-39/D6-13/JH5/VLκO12/2/JLκ1/κ2 (Set I), VH4-34/D5-5/JH6/VLκA17/JLκ1/κ2 (Set II), VH3-21/JH6/VLλ3h/JLλ3 (Set III), VH1-69/D3-16/JH3/VLκA27/JLκ1/κ4 (Set IV), VH1-69/D3-10/JH6/VLλ1c/JLλ1 (Set V), VH1-02/D6-19/JH4/VLκO12/2/JLκ1/κ2 (Set VIa); VH1-03/D6-19/JH4/VLκO12/2/JLκ1/κ2 (Set VIb); VH1-18/D6-19/JH4/VLκO12/2/JLκ1 (Set VIc); VH1-46/D6-19/JH4 (Set VId); VH5-51/D6-19/JH4/VLκO12/2/JLκ2 (Set VIe), VH1-69/D3-3/JH4/VLκA19/JLκ4 (Set VII), and V_H1-69/D2-2/J_H6/V_LκL6/2/J_Lκ3 (Set VIII). In some preferred embodiments, the family members of the light chain antibody gene and the heavy chain antibody gene are selected from the group consisting of

Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, and Set VIII; in other preferred embodiments, the family members of the light chain antibody gene and the heavy chain antibody gene are selected from the group consisting of Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VII. In additional preferred embodiments, the family members of the light chain antibody gene and the heavy chain antibody gene are selected from the group consisting of Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VIII. In still other preferred embodiments, the family members of the light chain antibody gene and the heavy chain antibody gene are selected from the group consisting of Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VII.

These preparations, comprising the antibody genes of each of the 12 identified sets, are useful for preparing reagents for diagnosis and treatment methods described below. Such useful reagents include compounds that specifically bind to the antigen binding site of the antibodies encoded by these genes, as further described below.

The antibody genes in these sets can be identified without undue experimentation by known methods, e.g., as described in the Example, using routine sequencing methods. The antibody genes are categorized herein as from a particular germline gene even if the antibody gene has several mutations.

The combination of antibody genes can be in any form, including single chain genes, as are known in the art. Preferably, the antibody genes are on a vector or vectors, such as a plasmid or viral vector, in order to facilitate their maintenance, as with a cloning vector, and to be able to produce the antibodies encoded by the genes, as with an expression vector. Cells in culture comprising a vector comprising antibody genes from Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII are also envisioned. Preferably, the antibody genes are selected from the group consisting of Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, and Set VIII, or Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VII, or Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VIII, or Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VII.

In other embodiments, the invention is directed to isolated and purified antibodies encoded by antibody genes from one of Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII. Preferably, the antibody genes are selected from the group consisting of Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, and Set VIII, or Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VII, or Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VIII, or Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VII. As previously discussed,

these antibodies, which are expressed as the B cell receptor on the B-CLL cells from individuals in the identified sets, can be used to identify reagents that bind to the antibody's antigen binding site. These antibodies can be produced by any known method. Non-limiting examples include antibodies from a hybridoma made from the CLL cells and antibodies from cloned antibody genes. As used herein, the antibodies can be in any form that includes at least one antigen binding region. The term "antibody" thus includes an Fab, Fab2, or Fv fragment. The present invention also includes hybridomas that produce the above antibodies.

As is known in the art, a consensus sequence for each set can be identified that provides the amino acid sequence that is most similar to the sequence of the antibodies of all members of the set. This consensus sequence can be used to identify an antibody binding site that is most similar to all the members of the set, in order to most efficiently produce a binding partner (e.g., an anti-idiotype antibody) that binds to all members of the set. Thus, the invention is also directed to these amino acid consensus sequences and to nucleotide sequences encoding the consensus sequences.

The invention is also directed to anti-idiotype antibodies that bind to the antigen-binding region of an antibody encoded the antibody genes of Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII. Preferably, the antibody genes are selected from the group consisting of Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, and Set VIII, or Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VII, or Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VIII, or Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VII. Since these anti-idiotype antibodies bind to the antibody binding site of the antibodies that are the B cell receptor of a B-CLL cells from a significant portion of B-CLL patients with the aggressive form of the disease, the anti-idiotype antibodies can be used in various diagnostic and treatment methods for B-CLL.

The anti-idiotype antibodies of these embodiments can be made by standard methods, e.g., screening a phage display library, or producing a hybridoma making monoclonal antibodies against the antigen binding site of the antibodies encoded by the various B-CLL gene sets described above. As such, these anti-idiotype antibodies can be from any vertebrate species but are preferably mouse antibodies, human antibodies, or humanized antibodies. Such antibodies can be made by known methods without undue experimentation. The present invention also includes hybridomas that produce the above anti-idiotype antibodies.

In related embodiments, the invention is directed to bispecific antibodies comprising the binding site of any of the above-described anti-idiotype antibodies and a binding site that binds to another B cell antigen. The B cell antigen can be any antigen on the B cell, such as a signal-

transducing antigen (either surface or intracellular), or a surface antigen. It is expected that, in many cases, the bi-specific antibodies having a binding site to a B cell surface antigen would bind to the B cell more tightly than an antibody with two anti-idiotype binding domains, since anti-idiotype antibodies can be of low avidity. The bi-specific antibodies having a binding site to a signal-transducing antigen would be expected to expedite the signaling pathway, such as a terminal differentiation pathway or an apoptotic pathway, thus expediting the elimination of a B cell contributing to the B-CLL disease.

The above anti-idiotype antibodies can also be combined in a mixture that provides the antibodies directed to the binding sites from more than one set. This mixture can include as many anti-idiotype antibodies as desired, including those any combination, or all of the sets. The latter mixture would be effective in diagnosis or treatment methods for all of the sets, rather than just one set.

When used for treatment methods, the above-described anti-idiotype antibodies or mixtures thereof would be in a pharmaceutically acceptable excipient.

The above-described anti-idiotype antibody compositions can be formulated without undue experimentation for administration to a mammal, including humans, as appropriate for the particular application. Additionally, proper dosages of the compositions can be determined without undue experimentation using standard dose-response protocols.

Accordingly, the compositions designed for oral, lingual, sublingual, buccal and intrabuccal administration can be made without undue experimentation by means well known in the art, for example with an inert diluent or with an edible carrier. The compositions may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the pharmaceutical compositions of the present invention may be incorporated with excipients and used in the form of tablets, troches, capsules, elixirs, suspensions, syrups, wafers, chewing gums and the like.

Tablets, pills, capsules, troches and the like may also contain binders, recipients, disintegrating agent, lubricants, sweetening agents, and flavoring agents. Some examples of binders include microcrystalline cellulose, gum tragacanth or gelatin. Examples of excipients include starch or lactose. Some examples of disintegrating agents include alginic acid, corn starch and the like. Examples of lubricants include magnesium stearate or potassium stearate. An example of a glidant is colloidal silicon dioxide. Some examples of sweetening agents include sucrose, saccharin and the like. Examples of flavoring agents include peppermint, methyl salicylate, orange flavoring and the like. Materials used in preparing these various compositions should be pharmaceutically pure and nontoxic in the amounts used.

In preferred embodiments, the anti-idiotype antibody compositions of the present invention can easily be administered parenterally such as for example, by intramuscular, intrathecal, subcutaneous, intraperitoneal, or, in the most preferred embodiments, intravenous injection. Parenteral administration can be accomplished by incorporating the compositions of the present invention into a solution or suspension. Such solutions or suspensions may also include sterile diluents such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents. Parenteral formulations may also include antibacterial agents such as for example, benzyl alcohol or methyl parabens, antioxidants such as for example, ascorbic acid or sodium bisulfite and chelating agents such as EDTA. Buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose may also be added. The parenteral preparation can be enclosed in ampules, disposable syringes or multiple dose vials made of glass or plastic.

Rectal administration includes administering the pharmaceutical compositions into the rectum or large intestine. This can be accomplished using suppositories or enemas. Suppository formulations can easily be made by methods known in the art. For example, suppository formulations can be prepared by heating glycerin to about 120° C., dissolving the composition in the glycerin, mixing the heated glycerin after which purified water may be added, and pouring the hot mixture into a suppository mold.

Transdermal administration includes percutaneous absorption of the anti-idiotype antibody composition through the skin. Transdermal formulations include patches (such as the well-known nicotine patch), ointments, creams, gels, salves and the like.

The present invention includes nasally administering to the mammal a therapeutically effective amount of the composition. As used herein, nasally administering or nasal administration includes administering the composition to the mucous membranes of the nasal passage or nasal cavity of the patient. As used herein, pharmaceutical compositions for nasal administration of a composition include therapeutically effective amounts of the composition prepared by well-known methods to be administered, for example, as a nasal spray, nasal drop, suspension, gel, ointment, cream or powder. Administration of the anti-idiotype antibody composition may also take place using a nasal tampon or nasal sponge.

In other embodiments, the invention is directed to peptide antigens that bind to the antigen-binding region of an antibody encoded by antibody genes from Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII. Preferably, the antibody genes are selected from the group consisting of Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, and Set VIII, or Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VII, or Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set

VIe, and Set VIII, or Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VII. Such peptide antigens can be made by well-known methods, e.g., phage display library or high-density peptide library, without undue experimentation.

As used herein, the term "peptide antigen" includes peptide mimetics, also known as peptidomimetics, which retain the same binding abilities as the analogous amino acid peptide. Peptide mimetics are peptides comprised of amino acid analogs, such as D-amino acids, that are more resistant to protease degradation than their L-amino acid peptide counterparts. Various peptide mimetics are known in the art, and any peptide mimetic can be produced without undue experimentation.

As is analogous with the anti-idiotype antibodies, these peptide antigens can be prepared as a mixture, in order to provide a diagnostic or therapeutic reagent useful for several, or all of the B-CLL sets. Also as with the anti-idiotype antibodies, the peptide antigens can also be usefully provided in a pharmaceutically acceptable excipient, for therapeutic applications, preferably for parenteral administration.

In further embodiments, the invention is directed to aptamers that bind to the antigen-binding region of an antibody encoded by antibody genes from Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII. Preferably, the antibody genes are selected from the group consisting of Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, and Set VIII, or Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VII, or Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VIII, or Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VII. As is known, aptamers are single stranded oligonucleotides or oligonucleotide analogs that bind to a particular target molecule, in this case an antibody binding site. Thus, aptamers are the oligonucleotide analogy to antibodies. However, aptamers are smaller than antibodies, generally in the range of 50-100 nt. Their binding is highly dependent on the secondary structure formed by the aptamer oligonucleotide. Both RNA and single stranded DNA (or analog), aptamers are known. Thus, these aptamers are analogous to the anti-idiotype antibodies and the peptide antigens previously discussed. As such, they can also be provided as a mixture of two or more, in order to have a reagent that can be utilized with more than one set of patients. They can also be provided in a pharmaceutically acceptable excipient, for therapeutic purposes, preferably for parenteral administration.

In some embodiments, the anti-idiotype antibody, peptide antigen, aptamer, or mixtures of these as previously described can usefully be functionalized or derivatized. One useful derivitization includes a cellular toxin. Such reagents are useful in a "magic bullet" approach to B-CLL therapy, where the toxin would be expected to kill only the B-CLL cell that the anti-

idiotype antibody, peptide antigen, or aptamer bound. Several cellular toxins known in the art for these embodiments can be used for this approach, including radioactive moieties, ricin, and chemotherapeutic agents.

In other embodiments, the anti-idiotype antibody, peptide antigen, aptamer, or mixtures of these as previously described can usefully be further functionalized to comprise a detectable moiety, such as a fluorophore, or an enzyme that can be treated with a substrate to produce a colored reaction product. Non-limiting examples of the latter enzyme is horseradish peroxidase and alkaline phosphatase. Such labeled anti-idiotype antibody, peptide antigen, aptamer, or mixtures can be used for diagnostic purposes, for example in labeling the B-CLL cells for fluorescence activated cell sorter analysis or for histological observation of the cells. These methods are more fully described below.

In additional embodiments, the invention is directed to multimeric molecules comprising at least a first and a second binding site, the first binding site binding to the antigen-binding region of an antibody encoded by antibody genes from Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII, and the second binding site binding to either (a) the same antigen-binding region of an antibody as the first binding site or (b) another B-cell antigen. Preferably, the antibody genes are selected from the group consisting of Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, and Set VIII, or Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VII, or Set II, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VIII, or Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, and Set VII. By providing multiple binding sites to a particular set, these multimeric compositions would be expected to bind more effectively than the single binding site peptide antigens or aptamers, or the double binding site anti-idiotype antibodies, as described above. In preferred embodiments, the multimeric molecules of these embodiments comprise more than five binding sites. These multimeric molecules can be made by the skilled artisan without undue experimentation.

In some embodiments, all of the binding sites of the multimeric molecule bind to the antigen-binding region of an antibody encoded by antibody genes from Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII. These binding sites can be directed to one epitope, to more than one epitope of the antigen-binding region, or to antigen-binding regions of more than one set.

In these multimeric molecules, the binding sites can be all antibody binding sites, all peptide binding sites, all aptamer binding sites, or combinations thereof.

More generally, the invention is further directed to an isolated and purified preparation of a combination of a light chain antibody gene and a heavy chain antibody gene, where the gene

family members of the light chain antibody gene and the heavy chain antibody gene are present in B cells of two or more patients, where the antibody chains of the B cells also share the same isotype, JH, D and JL regions, and where the B cells are lymphoproliferative in the patient, or where the patient has an autoimmune disease involving the B cells.

The discovery that B-CLL patients can be classified into sets having common antibody chains raises the possibility that other lymphoproliferative or autoimmune diseases involving B cells can also be classified into sets, where each set of patients share B cells that are involved in the disease with the same antibody genes. The instant disclosure provides evidence for this, since a patient in Set I has an immunocytoma, a patient in set II has a small cell lymphocytic lymphoma (SLL), and a patient in set VIa has a marginal zone lymphoma (SMZL) (FIG. 1). It is also highly probable that other B-CLL sets exist.

Preferred lymphoproliferative disorders within these embodiments include Hodgkin's disease, non-Hodgkin's lymphoma, Burkitt's lymphoma, myeloma, a monoclonal gammopathy with antibody-mediated neurologic impairment, a monoclonal gammopathy of unknown significance, and a monoclonal lymphocytosis of undetermined significance. Preferred autoimmune diseases within these embodiments include systemic lupus erythematosus, myasthenia gravis, Grave's disease, type I diabetes mellitus, autoimmune peripheral neuropathy, and autoimmune hemolytic anemia.

As previously discussed, the above compositions are useful for various diagnostic and therapeutic methods that are envisioned as part of the invention.

Thus, in some embodiments, the invention is directed to methods of

(a) determining whether a patient with B cell chronic lymphocytic leukemia (B-CLL) has a form of B-CLL susceptible to treatment directed to eliminating idiotype-specific B cell receptor-bearing B-CLL cells, or

(b) following the progression of treatment of B-CLL in a patient having a form of B-CLL susceptible to treatment directed to eliminating idiotype-specific B cell receptor-bearing B-CLL cells. In these embodiments, the methods comprise determining whether the B cell receptors on the B-CLL cells have an idiotype encoded by antibody genes from Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII. A determination that the B cell receptors have the specified idiotype at once establishes that the patient apparently has an aggressive form of B-CLL, and that the B-CLL can be treated using the anti-idiotype, peptide, aptamer, mixtures, or multimeric molecules described above, particularly those conjugated to a cellular toxin. Additionally, by continual monitoring of the idiotype of the B cells from the patient, one can follow the progress of treatment, since an effective treatment would exhibit a decreasing amount of B cells having an idiotype from the B-CLL set. No B cells having an

idiotype from the B-CLL set essentially means that the patient is in remission or cured of the B-CLL.

It can be seen, then, that it is useful to monitor progression of the treatment by quantifying the B cells having an idiotype from the B-CLL set, since a decreasing quantity of the B cells indicates an effective treatment, while an increasing quantity of the B cells indicates an ineffective treatment.

In these methods the determination step can be by any means known in the art. Nonlimiting examples include (a) amplification of idiotype-determining regions of the antibody genes or mRNA, e.g., by polymerase chain reaction, and evaluating whether the amplified regions are amplified from the B-CLL set in question; (b) sequencing the amplified regions; (c) evaluating whether the amplified regions hybridize with equivalent regions from the B-CLL set in question; (d) evaluating whether the patient has circulating antibodies with an idiotype encoded by the antibody genes from the B-CLL set in question; (e) evaluating whether the patient has antibodies that bind to a binding agent (e.g., an anti-idiotype antibody, a peptide antigen, or an aptamer, as described above, preferably comprising a detectable moiety) specific for the idiotype encoded by the antibody genes from the set in question; or (f) mixing a labeled anti-idiotype antibody, peptide antigen, or aptamer with lymphocytes of the patient and determining whether lymphocytes that bind to the composition are present, e.g., using a Coulter counter or a cell sorter.

The above methods can be used with a B-CLL patient at any stage of the disease, including in a pre-leukemic, early leukemic, frank leukemic state. Furthermore, the B-CLL cells can be obtained from the blood, the bone marrow, the spleen, and/or the lymph nodes, depending on the results of initial diagnosis and the stage of the disease.

The present invention is also directed to methods of treating a patient having B-CLL caused by B cells comprising antibody genes from Set I, Set II, Set III, Set IV, Set V, Set VIa, Set VIb, Set VIc, Set VId, Set VIe, Set VII, or Set VIII. The methods comprise administering to the patient the above described anti-idiotype antibody, peptide antigen, aptamer, or mixture as previously described, in a pharmaceutically acceptable excipient.

Although the anti-idiotype antibody, peptide antigen, aptamer, or mixture by themselves could be effective in eliminating the B cells, because they could set off an apoptotic cascade in the cells, it is preferred that the anti-idiotype antibody, peptide antigen, aptamer, or mixture also comprise a cellular toxin, as described above, that can directly kill the cell.

Additionally, the invention is directed to methods of identifying other B-CLL sets. The methods comprise identifying the VH, D, JH, VL, and JL classes of antibody genes present on B-CLL cells, where the same classes are all present in more than one B-CLL patient. It is

understood that databases and computerized comparison methods could be employed in this identification process.

Once additional sets are identified, a compound that binds to the antigenic site of an antibody encoded by the antibody genes can be identified by methods previously described, where the compound is useful for therapeutic and diagnostic purposes. Since the results provided in the Example establish that a significant proportion of B-CLL patients are in a set that shares the same B-CLL antibody genes with other patients, it is highly likely that other sets will be found.

It would be understood by the skilled artisan that the therapeutic agent in these methods is preferably an anti-idiotype antibody, a peptide antigen, or an aptamer that binds to the antigen binding site of the antibody encoded by the antibody genes that are typical of a 'set'.

Preferred embodiments of the invention are described in the following examples. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims, which follow the examples.

Example 1. Multiple Distinct Sets of Stereotyped Antigen Receptors Indicate a Role for Antigen in Promoting Chronic Lymphocytic Leukemia

Example Summary.

Previous studies suggest that the diversity of the expressed variable (V) region repertoire of the Ig H chain of B-CLL cells is restricted. Although limited examples of marked constraint in the primary structure of the H and L chain V regions exist, the possibility that this level of restriction is a general principle in this disease has not previously been known. This report describes eight sets of patients, mostly with unmutated or minimally mutated *IgV* genes, with strikingly similar BCR arising from the use of common H and L V region gene segments that share CDR3 structural features such as length, amino acid composition, and unique amino acid residues at recombination junctions. Thus, a much more striking degree of structural restriction of the entire BCR and a much higher frequency of receptor sharing exists among patients than previously appreciated. The data imply that either a significant fraction of B-CLL cells were selected by a limited set of antigenic epitopes at some point in their development and/or that they derive from a distinct B cell subpopulation with a limited Ig V region diversity. These shared, stereotyped Ig molecules may be valuable probes for antigen identification and important targets for cross-reactive idiotypic therapy. Sets II, IV, V, VI and VIII are described in Messmer et al., 2004, where they are named Sets IV, I, III, V and II, respectively.

Introduction.

The B-lymphocyte clone expanded in chronic lymphocytic leukemia (B-CLL) expresses low levels of surface membrane Ig, the B cell antigen receptor (BCR). The genetics of this Ig have clinical relevance, as patients with a clone whose Ig variable (V) region has no or few mutations have a significantly worse outcome than those with significant numbers of Ig V mutations (Damle et al., 1999; Hamblin et al., 1999). The biology underlying this association is unclear.

Several lines of evidence support a role for the BCR in the evolution of B-CLL (reviewed in Chiorazzi and Ferrarini, 2003). The distribution of individual *IgV_H* in B-CLL clones differs from that found in normal cells (Fais et al., 1998), with an increased frequency of *V_H1-69*, *V_H4-34*, and *V_H3-07* (Fais et al., 1998; Schroeder and Dighiero, 1994; Johnson et al., 1997). In addition, the distribution of mutations among B-CLL cases using these specific V_H genes is selectively biased (Fais et al., 1998; Schroeder and Dighiero, 1994; Kipps et al., 1989).

Recently two subgroups of B-CLL cases with remarkable similarity of the entire BCR (V regions of the H and L chain) were identified (Tobin et al., 2003; Ghiotto et al., 2004). Although these findings are provocative, they have been considered rare and potentially anomalous, since, in one instance the clones expressed IgG (Ghiotto et al., 2004) and in the other geography and ethnicity may be relevant (Tobin et al., 2002). This report describes another eight groups of B-CLL patients that express BCRs of strikingly similar primary structure defined by highly similar Ig V regions in the H and L chains and, in particular, distinct H and L CDR3 configurations. Thus, a significant fraction of B-CLL clones derive from B-lymphocytes with constrained antigen binding sites that could recognize individual, discrete antigen(s) or classes of structurally similar epitopes.

Materials and Methods

<u>IgV gene sequencing.</u> V_HDJ_H and V_LJ_L sequences were determined by previously described methods (Fais et al., 1998; Ghiotto et al., 2004).

<u>Database Searches.</u> B-CLL Ig H chain V amino acid sequences from our collection (n=255) and the public databases (n=197) were subjected to BLAST searches of both nucleotide and protein databases to identify similar sequences. The criteria used to define "Sets" of similar rearranged V_HDJ_H were: A) use of the same V_H, D, and J_H germline genes, B) use of the same D segment reading frame and position relative to the V_H, plus or minus one codon, and C) an amino acid similarity within the HCDR3 of ≥60% identity. In addition, all B-CLL Ig H protein sequences were aligned and clustered using the ClustalW alignment algorithm. Sequences clustering tightly were visually inspected for similarity. All of these searches used the complete V_HDJ_H and as such were weighted toward sequences that used the same V_H gene. To identify

sequences with similar HCDR3 but different V_H genes, CDR3 motifs from the various sets were used to search the public databases with the ProteinInfo search engine (http://prowl.rockefeller.edu/). The criteria for the members of Set V were altered to permit the use of different IgV_H genes that were members of the same IgV_H clan, while retaining the criteria for the rearranged V_LJ_L. Use of the same specific IgV_L gene and ≥85% LCDR3 identity was required for the inclusion of a companion rearranged V_LJ_L in a Set.

538 V_H sequences from $CD5^+$ and $CD5^-$ peripheral B-lymphocytes (Tobin et al., 2002; Geiger et al., 2000) were downloaded from the public database. These 538 sequences were independently compared to the translated databases using tblastn on the BlastMachine at the AMDeC Bioinformatics Core Facility at the Columbia Genome Center, Columbia University.

Detailed nucleotide and amino acid sequence alignments of the junctional regions and complete protein sequence alignments of the sequences described here are provided in the Figures.

Results and Discussion

Identification of subgroups of B-CLL patients with highly restricted V_HDJ_H segments and shared HCDR3 configurations. Each B-CLL-derived V_HDJ_H sequence in our database was compared with every B-CLL sequence in our collection (n = 255) as well as with those in the public Ig V gene databases (n = 197) using nucleotide and protein sequence BLAST. In addition, all available B-CLL H chain V region sequences were phylogenetically grouped using the ClustalW method; sequences that clustered together were further analyzed for HCDR3 sequence similarity. These screening methods identified Sets of sequences (Table 1) consisting of the same IgV_H with highly similar HCDR3 resulting from identical D (when identifiable) and J_H segment use, D segment reading frame, similar D segment position relative to IgV_H, and HCDR3 length, and significant (≥60%) amino acid sequence identity.

Table I. Sets of B-CLL cases that share Ig V region genes and have a high degree of similarity in H CDR3.

Set	Int. B-CLL[a]	Public B-CLL	Pub. other	Isotype	V_H	V_H Mutation %			D	J_H	V_L	J_L
						max	min	median				
I	5	0	1[b]	IgG	4-39	1.0	0.0	0.5	6-13	5	κO12/2 (4/5)	κ1/κ2
II	3	2	0	IgG	4-34	3.1	2.0	2.7	5-5	6	κA17 (3/3)	κ1/κ2
III	3	2	2[c]	IgM	3-21	2.4	0.0	1.4	ND	6	λ3h (4/4)	λ3
IV	2	2	1[d]	IgM	1-69	0.6	0.0	0.0	3-16	3	κA27 (2/2)	κ1/κ4
V	0	4	0	IgM	1-69	0.3	0.0	0.3	3-10	6	λ1-16 (1/1)	λ1
VIa	4	2	1[e]	IgM	1-02	0.3	0.0	0.0	6-19	4	κ O12/2 (4/4)	κ1/κ2
VIb	2	4	0	IgM	1-03	2.0	0.3	0.8	6-19	4	κ O12/2 (3/3)	κ1/κ2
VIc	1	0	0	IgM	1-18	1.2	1.2	1.2	6-19	4	κ O12/2 (1/1)	κ1
VId	0	2	0	IgM	1-46	0.0	0.0	0.0	6-19	4	0/0	
VIe	1	6	0	IgM	5-51	2.7	0.0	0.2	6-19	4	κ O12/2 (1/2)	κ2
VII	2	0	0	IgM	1-69	0.0	0.0	0.0	3-3	4	κ A19 (1/1)	κ4
VIII	3	0	0	IgM	1-69			0.0	2-2	6	κL6	κ3

[a] Internal B-CLL
[b] immunocytoma, accession Y09249
[c] small lymphocytic leukemia, accession AF299104, and elderly normal, accession AF174100
[d] anti-cardiolipin antibody, accession AF460965
[e] small marginal zone lymphoma, accession AJ487492

Three subsets of Set VI (VIa, VIb, and VIe) contained sequences that utilized different IgV_H genes but used the same D and J_H segments, the same $V\kappa$, and had highly similar HCDR3 configurations. Therefore, we used the HCDR3 motif common to these three subsets to search public databases for additional sequences with the same HCDR3 configuration potentially associated with a different IgV_H segment. This search was not restricted to B-CLL sequences. The approach confirmed the previously identified subsets and identified two additional subsets of Set VI (VIc and VId).

The public database searches identified 21 V_HDJ_H sequences, belonging to one of the eight individual Sets, bringing the total number of sequences among these Sets to 45. Interestingly, only two of the 21 sequences culled from the public databases were *not* derived from B-CLL cells. These two were from an anti-cardiolipin antibody producing B cell (Set IV) and from a splenic marginal zone lymphoma (Set VIa). This distribution of similar sequences is particularly striking since, at the time of this search, the public databases contained only 197 Ig H chain V region sequences from B-CLL patients (excluding those from our laboratories) out of a total of over 8,500 H chain V region sequences (search of Entrez with terms "human immunoglobulin heavy chain variable" produced 8,874 hits in the nucleotide database and over 6,183 hits in the protein database on 12/16/03).

Pairing restricted V_LJ_L rearrangements with V_HDJ_H segments in Sets. V_LJ_L sequences corresponding to the shared V_HDJ_H of the 5 Sets were available for most of our B-CLL cases and for a few of those identified in the public databases. Remarkably, the available IgV_L were highly conserved within Sets and the corresponding J_L were very restricted (Table 1 and FIG. 2). Six of the eight Sets with available L chains expressed the κ isotype.

IgV gene mutation status and isotype restrictions of individual Sets. Most of the IgV_H sequences in each Set differed by <2.0% from the most similar germline gene, with the exception of Set II in which the median level of mutation was 3.0 %. Notably, the deduced protein structures in those sequences that were considered "mutated" using the typical 2% threshold differed from the germline by relatively low levels. Only one sequence, from Set II (CLL ID47, FIG. 2), differed by more than 5% from its germline counterpart. The corresponding IgV_L in each Set exhibited low levels of mutation; in some cases V_L displayed <2.0% difference while V_H had ≥2% difference from the germline sequence (Table 1 and FIG. 2).

The H chain isotype was the same among members of a Set. All Sets expressed IgM, except for Set IV that consisted of IgG^+ cases, similar to a patient group reported previously (Ghiotto et al., 2004).

H and L CDR3 characteristics of the individual Sets. We identified trends in the chemical, structural, or functional nature of the residues that comprise the H and L CDR3s, and in particular their V_H-D and D-J_H junctions. For example, the *D* segments in the HCDR3s of these Sets were read in the hydrophobic and stop reading frames more often than in normal (Zemlin et al., 2003) and B-CLL (Fais et al., 1999) cells. For all cases in Set VI, the *D6-19* segment is read in a non-productive reading frame. However, the germline stop codon, located in the region of overlap with the terminal IgV_H sequence, was trimmed, allowing productive rearrangements with the J_H4 segment (FIG. 8).

Also of note was the repeated occurrence of certain non-germline encoded amino acids within *D* segments in some of the Sets. For example in all members of Set VIII, a change to M is found at the 3'end of the *D* segment (FIG. 5), a position that is not known to be polymorphic. Three of 7 sequences in Set V had an R to Q change within the *D3-10* segment that is also not listed as polymorphic (FIG. 6). In 4 of 5 cases in Set II, P replaced A in the portion of HCDR3 encoded by the canonical *D5-5* segment. While this is most likely a polymorphism of the *D* segment rather than a common mutation, the last of the 5 sequences in this set (CLL ID47) also deviates from the canonical *D5-5* sequence at this codon, substituting a D (FIG. 7). Thus even if these amino acid changes represent polymorphisms, their relative consistency within each Set suggests a selection for these residues.

Members of several Sets have common junctional residues that were not templated by any known germline gene segments and therefore presumably arose from trimming and/or addition during recombinational assembly. The sequences in Set IV all contain a pair of Gs at the V_H-D junction and an N at the D-J_H junction (FIG. 4). A very similar V_H-D junctional finding exists in Set VIII (FIG. 5). All sequences in Set II contain an aromatic residue at the V_H-D and a pair of basic residues (R or K) at the D-J_H junction (FIG. 7).

Other trends in the composition of the H and L CDR3s are found in the other Sets. These and the fine details of the nucleotide and amino acid sequences of the V_HDJ_H and V_LJ_L junctions for each Set are shown and discussed in the Supplemental data (see FIGS. 4-8).

Structural similarities of the BCR among members of the Sets. The deduced V_HDJ_H and V_LJ_L protein sequences for each member of the stereotyped Sets are presented in FIGS. 2 and 3. Because most members of the Sets use the same IgV_H, primarily in an unmutated form, associated with the same D and J_H segments and since these rearrangements are virtually always paired with an identical IgV_L that is restricted in its linked J_L, the primary structural features of the entire BCR of each Set are likely remarkably similar. Furthermore, the amino acid sequences of HCDR1, HCDR2, LCDR 1, and LCDR2 of members of the individual Sets are extremely similar, if not identical (e.g., Sets IV, V, VIII, and the Set VI subsets). In Set II, some amino acid differences exist in these regions due to somatic mutation.

These data indicate a much more marked constraint on the primary structure of the BCR in B-CLL than previously appreciated. They also indicate that this principle occurs in a sizeable number of patients. Collectively, ~12% (31 of 255: 22 from this study, 5 from our previous study (Ghiotto et al., 2004), and 4 that match another described set (Tobin et al., 2002; 2003)) of all of sequences in our internal laboratory B-CLL database and ~20% (27 of 131) of those with unmutated IgV belong to one of the eight stereotyped Sets described here or one of the two patient groups mentioned above (Tobin et al., 2002; 2003; Ghiotto et al., 2004). Approximately the same overall frequency (~12%) was encountered among the sequences from the public databases (21 of 197), although the proportion of the public B-CLL sequences that are unmutated was not determined. Most of the rearrangements in these Sets lack or have few somatic mutations, and even those whose V_H surpass the 2% threshold commonly used as the criterion to define significant IgV gene mutations (Fais et al., 1998; Schroeder and Dighiero et al., 1994) are only slightly above that level. This suggests that restricted BCR structure is primarily a feature of those patients with the worse clinical course and outcome (Damle et al., 1999; Hamblin et al., 1999). It appears that 1 of 5 B-CLL cases with unmutated BCRs fit into one of these defined Sets. Additional Sets will likely be uncovered as more Ig V region sequences are defined in B-CLL, and all unmutated cases may be similar to one of a discrete number of archetypal Sets.

Although Sets IV, V, VII, and VIII use unmutated *1-69*, they differ from previously described *1-69*-expressing B-CLL cases that have restrictions in specific *D* and J_H segments associations (Fais et al., 1998; Johnson et al., 1997). These differences include J_H (J_H3 vs. J_H6 in Set I), D (*D2* vs. *D3* family and *VκL6* with an extremely short LCDR3 in Set VIII), and L chain (λ vs. κ in Set V) gene use.

Initial studies that considered only IgV_H or V_HDJ_H (Fais et al., 1998; Schroeder and Dighiero, 1994; Johnson et al., 1997; Chiorazzi and Ferrarini, 2001) pointed toward limited structural diversity in the antigen-binding sites of B-CLL. However, our results are much more striking because of the remarkable similarity of the sequences within a Set and the virtual mathematic impossibility that this similarity arose by chance. If gene segment use in B-CLL was random, the probability of finding the same combination of V_HDJ_H and V_LJ_L segments in independent leukemic (or normal) B cells would be $>1 \times 10^{-6}$. Therefore, one would not expect to identify two B-CLL patients with BCRs comprised of the same V_HDJ_H/V_LJ_L until >1 million cases were analyzed. This calculation is conservative since it does not account for diversity at the V_H-D, D-J_H, and V_L-J_L junctions that can be quite extensive (potentially exceeding 1×10^{-9} and reaching 1×10^{-12}), although receptor editing and revision could limit these possibilities somewhat. Nevertheless, the level and frequency of BCR structural restriction in clusters of patients reported here is extraordinary and appears to be higher than any other B or T cell lymphoproliferative disorder reported to date.

Finding similar Ig H chain V region sequences by homology searches of the public databases is not, in itself, completely surprising because some IgV_H are expressed in a biased fashion and ~6,600 different V_H-D-J_H combinations can occur. Because the databases contain more than that number of Ig H chain V region sequences, identifying the same recombined gene segments is not improbable. When we analyzed 538 $CD5^+$ and $CD5^-$ B cell-derived H chain V region sequences, we identified many pairs of similar sequences and some groups of similar sequences. However these groups derived from B cells of diverse sources, as would be expected if the similarities were the product of random chance. In contrast, the similarity to a given B-CLL-derived sequence detected in our database comparisons arose almost exclusively from other B-CLL sequences (19/21) or other lymphoproliferative disorders (1/21), even though the entire database was searched. Only one identified sequence was from a non-B-CLL clone and that coded an autoantibody (Table I and FIG. 2). Although the proper normal B cell repertoire against which B-CLL clones should be compared remains an open question (Chiorazzi and Ferrarini, 2003), these results demonstrate that sequence sets of restricted cellular origin are not a generalized phenomenon in the public database.

Therefore, the development of B-CLL must involve B cell clones with restricted *IgV* and/or BCR structure. While it seems unlikely that the expression of particular BCR gene combinations could be the sole promoting factor for leukemogenesis, a strong inherent bias in gene segment association and V_HDJ_H/V_LJ_L pairing in the B cell population that gives rise to B-CLL cannot be formally excluded, especially since the cell of origin for B-CLL is still uncertain (Chiorazzi and Ferrarini, 2003). Although evidence exists in mice for biases in the recombination of particular Ig V gene segments prior to antigen experience (Seidl et al., 1997), the extent of restriction imposed by recombination biases at both the H and L chain V gene loci in those instances, especially at the V - (D) - J junctions, are not as severe as in the Sets described here. To our knowledge, there is no known subpopulation of human B cells in which the frequency of similar rearrangements, independent of antigen selection, is as great as among these B-CLL cases.

Therefore, antigen selection probably has a strong restrictive influence on the transformation of a normal B-lymphocyte to a B-CLL cell. A simple model would postulate that the transforming event is coupled with antigen specificity, i.e., an individual B-lymphocyte from a highly diverse population could bind and internalize a transforming agent (e.g., virus) via its BCR. Although this seems unlikely, such a mechanism has been implied for B-CLL (Mann et al., 1987).

Alternatively, antigen could be a promoting factor for transformation, selecting specific clones for expansion from an initially diverse population of B-lymphocytes and fostering their development to and in the transformed state (Chiorazzi and Ferrarini, 2003). This would be the case if the B-CLL–susceptible cell population were pre-selected for antigen-reactivity, and therefore BCR structure, by exposure to distinct antigens or classes of antigens during their development. These clones could differ among patients, especially if the selecting antigens were foreign or autologous and possibly polymorphic. From within these clonal expansions, one member could develop an initial transforming lesion that would promulgate the leukemogenic cascade independent of antigen.

Finally, the initial transforming events could occur at random within a diverse B cell population or a previously antigen-selected population, and the subsequent nurturing of the transformed clone to clinical B-CLL could require ongoing BCR engagement by antigen (Chiorazzi and Ferrarini, 2003). Recently, clonal expansions of B cells with phenotypic characteristics of B-CLL were found in normal elderly individuals (Rawstron et al., 2002; Ghia et al., 2004. The clinical relevance of these clones is not established. However, they may represent clones that have some of the genetic lesions of B-CLL but lack BCR specificities that would result in sufficient ongoing stimulus to mature them into clinical B-CLL.

The remarkable protein similarity of the entire BCR among members of each Set (FIGS. 2 and 3) suggests that they could recognize the same or similar antigens. While the nature of the antigen(s) cannot be directly deduced from the Ig sequences presented here, there are several reasons to suspect that they are autoantigens or carbohydrates possibly derived from bacterial or viral coats, or a combination of the two.

V_H1-*69* (Sets I, II, and III) and V_H3-*21* (previously described Set in Tobin et al., 2002; 2003) are enriched among rheumatoid factors (Silverman et al., 1988; He et al., 1995). V_H4-*34* (Set II) is used in every case of monoclonal cold agglutinin disease (Pascual et al., 1992) and in autoimmune conditions. Indeed the inherent autoreactivity of this V_H segment elicits a major inhibitory process by the immune system that keeps *4-34*$^+$ B cells from diversifying into high affinity, isotype-switched B cells (Pugh-Bernard et al., 2001). The anti-cardiolipin antibody identified as a member of Set IV implies that the other members of that Set may be specific for cardiolipin or DNA, since some antibodies to the former react with the latter (Kumar et al., 2003). In addition, restricted V_HDJ_H and/or V_LJ_L gene segments are features of B cells that produce anti-carbohydrate mAb in human (Scott et al., 1989) and mouse (Potter, 1977).

Characteristic junctional residues are also a feature of anti-carbohydrate mAb and autoantibodies and basic junctional residues, as seen in Sets II, IV, and VIe (FIG. 2), often indicate reactivity with acidic targets such as DNA (Radic and Weigert, 1994). The synthesis of autoreactive Ig/BCR molecules by many B-CLL clones (Sthoeger et al., 1989; Borche et al., 1990) supports a link between the unique BCR structural features of these Sets and autoantibodies.

The non-B-CLL Ig sequences that matched these B-CLL stereotypes may give insight into the identity of the B-CLL progenitor cell(s). One of those two derived from a splenic marginal zone lymphoma (SMZL; Set VIa, FIG. 2) and the other from an autoantibody-producing B cell (Set IV, FIG. 2). Interestingly, normal MZ B cells produce mAb that can recognize thymus-independent type II antigens and autoantigens (Bendelac et al., 2001). In addition, the Ig V region repertoire of murine MZ B cells is very restricted in gene segment use and structure that requires intact BCR signal transduction to develop (Martin and Kearnet, 2000). MZ B cells appear to be progenitors for gastric MALT lymphoma (Isaacson, 1999) and have been proposed as precursors of B-CLL cells (Chiorazzi and Ferrarini, 2003). If one infers common antigenic reactivity based on the similar sequences within a Set, a significant fraction of B-CLL cases, and in particular those with unmutated *IgV* genes, produce mAb that recognize one of a limited, discrete array of antigens or epitopes. With such an interpretation, some B-CLL cases may resemble gastric MALT lymphoma regarding the role of antigenic drive (in that instance, *H.*

pylori) in the promotion of malignancy. The stereotyped Ig molecules reported here might be valuable probes to identify antigens that drive the leukemogenic process in B-CLL.

Finally, these Sets of stereotyped Ig molecules may serve as therapeutic targets on B-CLL cells. A conceptual drawback to targeting the BCR as a tumor-specific antigen has been the apparent need to create an individualized reagent for each patient. However, since our data indicate that there is potentially extensive overlap in BCR structure and specificities among groups of B-CLL cases, this approach may be far less daunting. Indeed, since ~20% of the cases with unmutated IgV_H genes fall into one of these Sets, such targeting might be most effective in those cases that have the worst prognosis, are least responsive to therapy, and have the most aggressive clinical courses (Damle et al., 1999; Hamblin et al., 1999).

In view of the above, it will be seen that the several advantages of the invention are achieved and other advantages attained.

As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

All references cited in this specification are hereby incorporated by reference. The discussion of the references herein is intended merely to summarize the assertions made by the authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinence of the cited references.